REACTIONS OF AN ELASMOBRANCH (*SQUALUS SUCKLII*) TO VARIATIONS IN THE SALINITY OF THE SURROUNDING MEDIUM.

J. P. QUIGLEY.

From the Pacific Biological Station, Nanaimo, B. C., and the Department of Physiology and Pharmacology, University of Alberta, Edmonton, Alberta.

A study of the changes which occur in fish when placed in a medium other than their natural habitat has attracted the attention of many investigators. Variations have been made both in the concentration and in the kinds of salts used and experiments of this nature have been conducted with teleosts and elasmobranchs. Our knowledge of the subject is, however, by no means complete, and a further investigation of certain phases was thought to be of value.

It is generally appreciated that elasmobranchs are usually less resistant to changes in salinity than are the teleosts. The former, therefore, make excellent subjects for an investigation of certain phases of the problem. *Squalus sucklii* was selected for the following investigation. This is a species of elasmobranch which has heretofore received little attention from physiologists.

In addition to observing the length of time this fish was able to live in certain solutions differing in amount and kind of salinity from its natural habitat an investigation was made of the changes in the gill movements, the heart beat, the amount of hemoglobin and the weight of the animal when taken from sea water and from the experimental solutions. A comparison was also made of the weight of the liver, spleen and pancreas taken from fish that had died in the various media.

The fish used in this research were taken on a set line from the Strait of Georgia near Departure Bay, Vancouver Island, and usually from a depth of 30 yards. The fish were handled very carefully but some injury always resulted from this method of capture. However, good specimens usually would live for a week when kept in cages fastened to a float in sea water despite the fact that they were not fed during this time and that the salinity of this water was lower than that of their natural habitat. The fish were taken during the months of June, July and August of 1926 and a total of 219 were obtained suitable for this investigation. The fish were kept in a cage fastened to a float in sea water and were usually used within a few hours after being taken from the set line, and always within 15 hours of the time of capture.

For the purpose of comparison some physical characteristics are given of the solutions with which this investigation is concerned (Table I.).

Methods.

In this investigation, the following media were used: (*a*) distilled water, (*b*) tap water, (*c*) tap water which had been given a pH of 8.4 by the addition of NaOH solution, (*d*) NaCl, in distilled water, (*e*) NaCl, $CaCl_2$ in distilled water, (*f*) NaCl, $CaCl_2$, KCl in distilled water, (*g*) NaCl, $CaCl_2$, KCl, $MgSO_4$ in distilled water, (*h*) sea water to which NaCl, $CaCl_2$, KCl, $MgSO_4$ had been added. In each case the quantity of the salt added was the amount required to give a concentration of the cation approximately the same as that found in sea water. When distilled water was used it was freshly made, cooled and then shaken with air. The water was obtained from a copper still but this is considered to be of negligible importance. The various solutions were frequently agitated during the course of the experiment and the fish were changed to a fresh medium of the same kind if the experiment continued for more than an hour.

Since it was desired to have conditions approximate as nearly as possible those which a fish would encounter if it were to swim naturally into the solution used, the fish were not "wiped." Before being placed in the solution they were allowed to hang head downward for one half minute, and then weighed. A slight amount of bleeding was produced by introducing the point of a scalpel having a thin blade into the caudal artery at the base of the caudal fin. Samples of blood were taken for the determination of the amount of hemoglobin and then the fish were placed in the desired solution.

TABLE I.

Medium.	pH.	Temperature Degrees C.	Density.	Total Solids gm. per Liter.	Oxygen Content cc. per Liter.	Grams per Liter.			
						NaCl	$CaCl_2$	KCl.	$MgSO_4$.
Water from which fish were captured	8.4	10.3 [1]	1.0218 [1]		4.41 [1]	27.37 [1]			
Water in cage where fish were kept	8.4	16–18	1.0184 [1]	28.72	5.43 [1]	22.17 [1]			
Distilled water	7.0	16–18	1.0000	0		0	0	0	0
Tap water	6.9	16–18	1.0003 [1]	0.087	5.32	Trace			
Tap water pH 8.4	8.4	16–18							
NaCl solution	7.0	16–18		27.0		27	0	0	0
NaCl, $CaCl_2$ solution	7.0	16–18		28.164		27	1.164	0	0
NaCl, $CaCl_2$, KCl solution	7.0	16–18		28.914		27	1.164	0.75	0
NaCl, $CaCl_2$, KCl, $MgSO_4$ solution	7.0	16–18		35.414		27	1.164	0.75	6.5
						Added to sea water			
Sea water with added salts	7.0	16–18		64.134		27	1.164	0.75	6.5

[1] C. C. Lucas, personal communication.

It is well recognized that the effectiveness of a stimulus is partly dependent on the rate at which it is applied, therefore the sudden immersion of the fish in the solution should result in a maximum effect. Also, the injuries which the fish received incident to capture might alter slightly the results of an experiment.

After a variable length of time, the respiratory movements became slower and weaker. As the paralysis of the respiratory movements became more marked some struggling usually occurred and finally respiration completely failed. When respiration ceased, as was evidenced by cessation of movements of the spiracle, the time was noted, the fish was removed from the water, suspended head downward for one half minute as before, and reweighed. Blood was again taken for the determination of hemoglobin. The fish was placed on a tray, the heart exposed and if found beating, as was practically always the case, the time when the cardiac movements ceased was noted. The liver, spleen and pancreas were then exposed, freed from adjacent tissue and weighed.

Observations were made of the respiratory movements of all the fish used while in the various solutions but a series of thirteen fish were used in making a more complete study of the effect of tap water on the nature of the respiratory and cardiac movements. Freshly caught fish were weighed and then fastened to a wooden frame. The frame was submerged in a tank of sea water in such a manner that all of the fish, with the exception of the ventral surface directly over the heart, was covered by the water. A median incision was made in the exposed area, the tissue at the sides of the incision was retracted in such a manner that the heart was exposed, but the entrance of water to the wound was prevented. By means of a thread, the apex of the ventricle was attached to a heart lever arranged to write on a kymograph drum. A second heart lever was attached to one of the gill slits and arranged to record respiratory movements on the same drum. A record was made of the respiratory and cardiac movements of the fish in sea water. This water was then quickly drawn out of the tank and replaced by tap water and a second record was made. After the fish had been in tap water for thirty minutes, a third record was made and then the water was replaced by fresh tap water. A fourth record was made after the fish had been in the tap water for fifty-five minutes. After the fish had been in the tap water for sixty minutes, it was removed from the water and killed by destruction of the central nervous system.

In determining the normal weight of the liver, spleen and pancreas 103 fish (34.9 per cent. females) of various sizes were used. These were fish that died in sea water either shortly before lifting the set line or soon after being placed in the cages. The fish were held head downward for one half minute to drain and then weighed. The abdominal cavity was opened by a median incision. The liver, spleen and pancreas were each dissected from the adjacent tissue and immediately weighed. From the figures so obtained, the weight of each organ per gram of fish was calculated.

Results and Discussion.

Toxicity of Media.—As a result of the studies of many investigators [for a review of this subject see Garrey (1, 2), Scott (3, 4), Macallum (5)] it has been shown that the osmotic pressure of the blood of a fish is rarely the same as that of its natural habitat. This is made possible by the relative impermeability of the integument, alimentary tract mucosa and of the gill membranes. The kidneys also play an important rôle in keeping the blood composition constant. However, when the fish is transferred to a medium having a different osmotic pressure the impermeability of the gill membranes is reduced and a passage of water and salts occurs which can not be entirely counter-balanced by the activity of the kidneys. This leads to a partial equalization of the osmotic pressures of the solutions separated by the gill membranes. In general, this equalization occurs more rapidly and more completely in elasmobranchs than in teleosts. Toxicity of a solution appears to some extent to be related to the degree to which it differs in osmotic pressure from that of the natural habitat of the fish. Therefore, teleosts will frequently survive changes in osmotic pressure of the external medium which would be fatal to elasmobranchs.

Distilled water has usually been found to be very toxic for fish, Ringer (6), Wells (7), but Garrey (2) found that fish can live in this medium for weeks and he quotes a similar observation made by Loeb. Garrey (2) has likewise corroborated the findings of Ringer (6) (8) that the toxicity of distilled water is reduced by the addition of a small amount of salts; for example, fresh water is less toxic than distilled water.

Many physiologists Loeb (9), Ringer (6), Garrey (2) have demonstrated with a number of species of marine and fresh water teleosts the mutual antagonism and progressive decrease in toxicity as Ca, then K, and finally Mg ions have been added in definite proportions to NaCl solutions. The usual explanation offered is that these ions exert their protective action by reducing the injurious effect which the individual ions have on the permeability of the gill membranes to salts and water.

In so far as I have been able to ascertain no previous investigation has been made on the effects of physiologically unbalanced solutions, with the possible exception of fresh water, on any elasmobranch or the effect of physiologically balanced solutions on *Squalus sucklii.*

In determining the effect of unbalanced, partially balanced and completely balanced solutions on dogfish it was found, that although the differences in the toxicity of the solutions used were not very great (Table II.), the results in general agreed rather well with those obtained by the other investigators. Using the time required for failure of the respiration as a guide to the toxicity, the solutions in order of their decreasing toxicity are Na > distilled water > Na, Ca, K, Mg, > Na, Ca, > Na, Ca, K. Since the solution containing the Na, Ca, K, and Mg ions was in substance an artificial sea water, it is difficult to explain its apparently high toxicity.

Using failure of the heart as a guide to the toxicity, the results are distilled water > Na > Na, Ca > Na, Ca, K, Mg > Na, Ca, K. The solution made by adding NaCl, $CaCl_2$, KCl, and $MgSO_4$ to sea water so that the cations would be present in approximately twice their normal concentration was a balanced solution. Therefore it is interesting to note that this solution as regards its effect on respiratory or cardiac movements was the

TABLE II.

Solution Used.	Number of Fish Used.[1]	Ave. Duration of Movements (in Minutes).		Ave. Change in Hemoglobin Gm. per 100 cc. Blood.	Ave. Percentage Change in Weight.
		Respiratory.	Cardiac.		
Distilled water	24	73	100	−0.613	+1.75
Tap water	23	113.2	144.7	−0.323	+3.66
Tap water pH 8.4	4	87.5	128	−0.487	+6.7
NaCl solution	10	71	137.7	−0.046	−1.09
NaCl, $CaCl_2$ solution	5	89	138	−0.035	−0.132
NaCl, $CaCl_2$, KCl solution	7	125	165	−0.0057	−2.379
NaCl, $CaCl_2$, KCl, $MgSO_4$ solution	6	88.3	153.3	+0.108	−0.032
Sea water with added salts	6	53.3	83.3	+0.345	+3.46

[1] Does not apply to determination of hemoglobin.

+ Indicates increase, − indicates decrease.

most toxic solution used. Experiments of Loeb and Wasteneys (10), Garrey (2) and Portier and Duval (11) also indicate that a balanced solution may be toxic when the concentration exceeds a certain limit.

The water referred to as tap water was a ground water caught in a small private reservoir and supplied to the Pacific Biological Station. It may be considered as similar to that which fish would encounter if they were to swim to a point above tide water in the streams around Departure Bay. This water had a very low salinity but it is quite possible that the cations Na, Ca, K and Mg were all present.

Tap water was found to require a longer time to produce cessation of respiration or cardiac failure than any of the experimental solutions with the exception of the Na, Ca, K solution, an observation which would strengthen the conclusion that toxicity is not a simple question of osmotic pressure. Since the dogfish continued to breath for an average of 113 minutes in tap water and remained active during most of this time, they probably could escape from a fresh water stream even if they were to swim into it above tide water level. As in the case of the salmon, Greene (12), the resistance of the dogfish to fresh water might be increased if it were to ascend the stream very gradually.

Scott (3), however, has shown that dogfish may be permanently injured by solutions whose osmotic pressure is markedly different from that of the normal habitat. This investigator has shown that when specimens of *Mustelus* were placed in either a hypotonic or hypertonic solution, the freezing point of the blood returned to normal either slowly or incompletely after being returned to sea water depending on the length of time the fish remained in the abnormal solution and the degree to which this solution differed from normal sea water. A similar conclusion may be drawn from the results obtained in this investigation for it was observed that after being left in distilled water or tap water until a disturbance of the respiration was observed, the condition of *Squalus sucklii* did not improve when transferred to sea water. The specimens of *Mustelus* used by Scott (3) in nearly every case died in less than 100 minutes when immersed in fresh water. Although it is likely that the tap water used in the experiments here described contained less salts than the fresh water used by Scott, *Squalus sucklii* (Table II.) continued to breath for an average of 113.2 minutes and cardiac failure occurred after 144.7 minutes.

Chidester (13) quotes the work of many investigators, from which it is to be concluded that fish are very sensitive to changes in the pH of the medium and, moreover, that the toxicity of a solution is less, the nearer it approaches the pH of the natural habitat. It was therefore considered of interest to determine whether the toxicity of tap water (pH 6.9) would be decreased by making the concentration of the hydrogen ion correspond with that of the normal sea water. For this purpose, NaOH was used rather than Na_2CO_3 so that the variation in the salinity would be slight. Contrary to expectations it was found (Table II.) that the water at pH 8.4 was more toxic than at 6.9. As will be shown later, the toxicity of abnormal media appears to be related to a depression of the respiratory center. It is therefore possible that the fish lived longer in the tap water pH 6.9 because this solution, being of an acid nature, was less depressant to the respiratory center than the tap water pH 8.4. It is also possible that the NaOH exerted a toxic action in some manner other than through an alteration of the pH. As will be shown later, the fish in this solution underwent a marked gain in weight, a fact which would indicate that the solution was very injurious to the gill membranes.

It was impossible to demonstrate any relation between the size of dogfish and their resistance to changes in salinity. Similar reports have been made for teleosts by Young (14) but Bert (15) maintained that the larger fish are more resistant. The fact that most of the large females used by the author were pregnant may be used in explaining why these fish were not more resistant.

Bert (16) has reported that when fresh water fish were transferred to sea water, circulatory changes occurred in the blood vessels of the gills so that the appearance of these structures was decidedly changed. In my comparison of the gills of dogfish that had died in sea water with those of fish that had died in the experimental solutions no constant variation in the appearance was observed.

When the dogfish were placed in the experimental solutions their behavior was in all cases, much the same. They usually remained quiet, giving no indication that the medium was obnoxious to them, but they moved energetically when disturbed. A white slimy material collected in the water and on the bodies of the fish after they had been in the solution for some time, and in some cases opisthotonos, most marked in the region of the neck, was observed. Shortly before and after the respiratory movements ceased, struggling movements frequently occurred. The eyes were usually opaque by the time respiration ceased. The significance of the previously mentioned slime is not known, but it may indicate that the solutions had an action on the integument of the fish, perhaps stimulating secretion from the gland cells of the integument. The formation of slime in teleosts associated with changes in the environment has been reported by Young (14) and by Bert (16).

Many pregnant fish were used in the experiments but in no case did abortion occur as the result of introducing the fish into the experimental solutions. On opening the abdominal cavity of these fish, after cessation of the heart beat, the embryos were in all cases found to be dead. Several pregnant fish taken directly from sea water were killed by destruction of the central nervous system. They were allowed to remain undisturbed for an hour or more and then the embryos were examined. In most cases they were found to be alive and active. The cause of death in embryos following the introduction of the mother into an abnormal medium is not known. Asphyxia and dilution of the blood probably play minor rôles in this phenomenon.

Gill Movements.—A gradual decrease in rate and amplitude of the respiratory movements followed the introduction of *Squalus sucklii* into a medium of abnormal salinity. Until the fish had been in the medium for about half the time they survived the change this decrease was usually slight and in some cases was absent, but during the latter half of the experiment the decrease in both rate and amplitude was more rapid.

For thirteen fish in sea water, following exposure of the heart, the respiratory rate varied between 18 and 73 but averaged 41.6 to the minute. The fish gave little or no evidence of injury as the result of the operation and the results obtained are believed to approximate rather closely those to be observed in the normal intact animal. One hour after placing the fish in tap water, the respiration of five had ceased and the respiratory rate of the remainder averaged 31.7 per minute.

Lyon (17) reports that the normal respiratory rate of the shark under experimental conditions varies between 18 and 30 per minute with an average rate of 23. A specimen of *Mustelus* examined by Scott (3) had a respiratory rate of 59 per minute just as the change from sea water to fresh water was being made. After sixty-seven minutes in fresh water this fish made only 8 very feeble respiratory movements per minute. A specimen of *Squalus acanthias* observed by the same investigator breathed while in sea water at the rate of 14 times per minute. Greene (12) states that the respiratory rate of the salmon varies between 60 and 120 per minute. Parker (18) records 35 to 40 respiratory movements in the normal specimen of *Mustelus* resting in sea water and 50 to 55 per minute in the fish swimming slowly.

Scott (3) noted that the respiratory and the heart rate in *Mustelus* were at times equal but they appeared to be little correlated. Lyon (17) found evidence to indicate that the heart of the sand shark normally takes its rate from the respiration. In the present investigation it was observed that in *Squalus sucklii* the respiratory rate and cardiac rate seemed to be unrelated (except as considered later, when struggling or gill cleaning movements occurred). They were rarely equal and usually the respiratory rate was between two and three times as rapid as the heart rate, except shortly before the death of the animal when the respiratory rate was markedly decreased while the heart rate had changed but little.

Hyde (19, 20) has noted slightly convulsive movements of the gills occurring in the normal skate and suggests that these may be for the purpose of forcing more water through the gill apertures and thus removing foreign matter. Lyon (17) observed that such movements occur in the shark when any foreign body or solution enters the mouth or when a manipulation of the body occurs. Scott (3) found that such movements became very marked in *Mustelus* some time after being transferred to fresh water but decreased in intensity and frequency before the death of the fish. He intimates that the injurious action of the fresh water on the gill membranes may be the cause of the increase in intensity and frequency of these movements. The gill cleaning movements did not occur according to Scott (3) when similar experiments were carried out on *Squalus acanthias*.

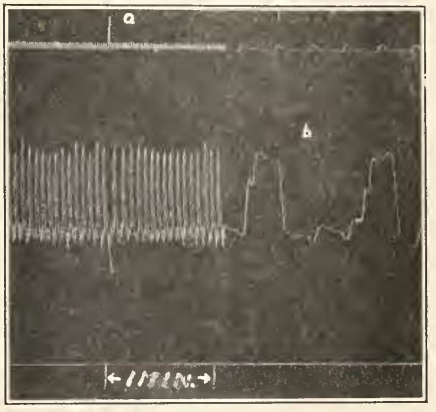
Movements similar to those described were noted in *Squalus sucklii*. These appear to be a normal movement and were observed while the fish were in sea water or in one of the experimental solutions. A more detailed study was made of these movements as they occurred when the fish were in tap water. With *Squalus sucklii* in tap water the gill cleaning movement may begin during any phase of the respiratory movement and consists of a single or more rarely two or three vigorous movements of the gills; the normal rhythm and amplitude is then regained. There is no preliminary movement preceding the gill cleaning movement. In some fish the gill cleaning movements may not be observed for some time but usually they occur at fairly regular intervals of one or two minutes. With certain fish, the intervals between these movements decreased to reach a minimum about one half hour after immersion in tap water and then decreased in frequency until respiration ceased. This was not of constant occurrence, for cases were observed where the movements disappeared after the immersion of the fish in tap water. The amplitude of the gill cleaning movements was not increased by immersion of the fish in fresh water. In fact it could not be definitely stated that immersion of the fish in fresh water produced any constant variation in these movements.

During the ventricular diastole following the gill cleaning movement, or more rarely during the second ventricular diastole, the heart apparently loses tone and marked dilatation occurs, the heart loses a beat and then quickly resumes the normal tone and rate. Scott (3) observed a similar change in *Mustelus* and suggested that "the cardiac spasm" is an instance of reflex inhibition of the heart beat due to the cardiac inhibitory center being stimulated by impulses from the sensory nerves. Reflex cardio-inhibition does readily occur in fish, Lyon (17), Greene (12), but because of its rhythmic nature and for other reasons the explanation of Scott does not appear to apply to the movements which I observed in *Squalus sucklii.* I am, however, in accord with his conclusion that the respiratory convulsions do not produce the peculiar cardiac movements, but that the two processes have the same cause.

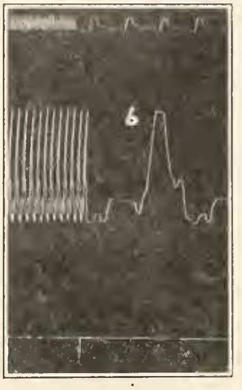
Distinction should be made between the movements described above and a somewhat similar movement observed in *Squalus sucklii.* This consisted of a spasm or series of struggling movements of the skeletal muscles but also involved the heart and gills. They appeared to occur during any part of the interval during which the fish were in the solutions and to originate in any external stimulus; occurring usually when the fish was touched or the water agitated. Several fish in a tank would remain quiet for a long interval, then a sudden movement of one individual would usually result in struggling movements among the others. These movements were more easily initiated after the fish had been in the solution for some time and especially just before and after respiration had ceased, that is, when the oxygen want might be expected to be greatest. The effect of these movements on the cardiac and respiratory movements was more pronounced but otherwise similar to those described as gill cleaning movements.

Although these movements would be likely to have a gill cleaning effect, this probably was not their primary object. They appeared to be the response given by the fish to a sensory

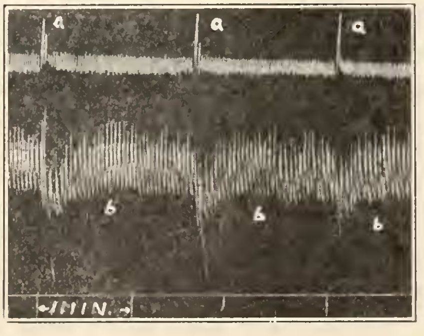
Simultaneous Records of Respiration (upper line) and Heart Beat (middle line).



Fish in sea water, (*a*) gill cleaning movement without a tonus wave in the record of the heart beat, (*b*) record on rapid drum.



After fish had been in tap water for 54 minutes, (*b*) record on rapid drum.



Record from fish after 6 minutes in tap water, (*a*) gill cleaning movements with (*b*) tonus waves.

stimulus after an increase in excitability had occurred. This increase in excitability may have been the result of the accumulation of CO_2 in the tissues due to the asphyxia which followed the introduction of the fish into an abnormal medium. Bert (16) has described the agitation and irregular respiration occurring in fresh water fish transferred to sea water and Young (14) has observed that when fresh water teleosts were placed in a solution whose salinity differed from that of their natural habitat the fish were restless and easily excited before death. Wells (21) has noted a somewhat similar condition in fish dying in a solution containing a high concentration of carbon dioxide and a low concentration of oxygen. This apparently is the same condition as that described above for the dogfish. No other forms of irregularities in the respiration of the dogfish were noted. Cheyne-Stokes rhythm is at times observed in cases of anoxemia, I therefore expected that it might occur while the fish were in the experimental solutions but it was not observed. Lyon (17) has noted this type of respiration occurring in the shark shortly before death.

Cause of Death of Fish in Experimental Solutions.—Perhaps the most noticeable change which occurred in the dogfish when placed in the experimental solutions was a gradual but progressive slowing of the respiration terminating finally in a complete cessation of the respiratory movements. Since this change occurred with all of the solutions used, it does not appear to be the result of the presence or absence of any of the salts used nor of the pH of the solution. The rate at which failure of respiration becomes complete as shown in Table II. does vary with the salt or combination of salts used. This has previously been considered.

Many investigators studying the effect of variations in salinity have noted the injurious effect on the respiration and Lyon (17) has noted that the respiration ceases before the heart beat when sharks die in sea water, while Greene (12) from a study of the salmon concludes that the spawning act produces death through some more vulnerable channel than the heart and blood vessels. It may be a rule of more or less general application for fish that the respiratory apparatus succumbs more readily than the circulatory.

Assuming that the fish used in our experiments died from asphyxia, it is interesting to note that the solutions used contained about as much oxygen at the beginning of the experiment as did the sea water from which the fish were captured. The solutions were agitated frequently and it is not likely that any marked decrease in the oxygen content occurred during the experiment. Moreover Powers (22) has noted that the oxygen content of the normal medium in which fresh water or marine fish live can be decreased to a low level (between 1.7–0.4 cc. per liter) before the fish will exhibit oxygen want.

That anoxemia was an important factor in the death of the fish placed in experimental solutions has been the conclusion of many investigators. Indeed, it has been shown by Backman (23) that if the sea water in which dogfish live, with $\Delta = -1.88^{\circ}$ is diluted to $\Delta = -0.5^{\circ}$, fifteen minutes after being placed in this solution, the tension of oxygen in the blood is diminished from 19.1 to 3.7 per cent. This anoxemia was believed to be a result of injury to the gill membranes and to the change in the water and salt content of the blood with the attendant change in size and the destruction of the blood corpuscles, thus decreasing the gas carrying power of the blood.

However, if fish were dying from asphyxia due to an injury of the gill membranes while the irritability of the respiratory center remained normal, hyperemia and dyspnoea might be expected. They did not occur in *Squalus sucklii*. The changes observed correspond more with those to be expected from a depression of the respiratory center.

As a result of this investigation and the contributions to this problem made by others, the author is inclined to explain the death of fish in media having an abnormal salinity to a progressive depression of the respiratory center. Although a change in the composition of the surrounding medium frequently alters the permeability of the limiting membranes of the body, the changes in the osmotic pressure of the blood and the injury to the gills, provided they occur, are considered to be only a contributary cause of death. The action of the abnormal medium on the respiratory center and perhaps on the motor nerves supplying the gills appears to be the primary cause of death. Changes in permeability of the limiting membranes are of importance in this regard insofar as they alter the activity of the structures mentioned by (*a*) making it possible for salts to diffuse into or from the inner ear and brain cavity, (*b*) initiating a reflex over some cutaneous nervous structure (lateral line?), (*c*) altering the composition of the blood supplying these structures. The relative importance of these factors may, of course, be subject to variations. According to this concept, teleosts are more resistant to changes in salinity, either because the changes mentioned above occur less readily or because the fish are more resistant to the changes after they do occur. In support of mechanism (*a*) we may consider the suggestion of Loeb and Wasteneys (10) that toxic salts ($NaNO_3$, NaBr, KCl) in the external medium exert their action in this manner. Furthermore, it is to be expected that a disturbance of equilibrium might be concomitant with changes in the inner ear. This phenomenon has been observed shortly before death when fish were placed in solutions of abnormal salinity, Loeb and Wasteneys (10) and Young (14), in solutions of glycerine, Siedlecki (24), and in solutions of low oxygen and high carbon dioxide content, Wells (21). Maxwell (25) states that in most selachians the lymph of the vestibule is in free communication with the exterior sea water through the ductus endolymphaticus, and that it is reasonable to suppose that the density of the lymph would be practically equal to that of sea water. Since this condition is never encountered in teleosts it may be that we have here an important factor in the different susceptibility of these two groups of fish to variation in salinity.

Cardiac Movements.—Observations of the heart beat of *Squalus sucklii* were confined to fish in sea water, tap water and fish that were removed from one of the other experimental solutions following cessation of respiration. In general the heart is much more resistant to changes in the external media than is the respiration.

The heart rate of fish in sea water, taken shortly after exposure of the heart varied between 14 and 31 beats per minute with an average of 18.9.

In the experiments where records were made of the cardiac movements while the fish were in tap water very little change in the beat occurred. The fish were removed from the tap water at the end of an hour, or earlier in those cases where the respiration ceased in less than an hour. No failure of the cardiac movements occurred during this interval. No irregularities nor loss of tone of the heart muscle occurred other than those changes associated with the gill cleaning and struggling movements. A slight decrease in the amplitude of the contractions did occur and the number of beats per minute at the end of the hour varied between 4 and 28 with an average of 14.6.

Scott (3) records a heart rate of 50 per minute in a pithed specimen of *Mustelus canis* immersed in sea water. After the fish had been in fresh water for an hour the amplitude of the beat was about the same as at the beginning but the rate was only one fourth as great. When the respiration failed the heart beat was forcible and strong but it failed rapidly after this.

This investigator also records that in a pithed specimen of *Squalus acanthias* placed in the same medium from which it was captured (brackish water) the rate was 16 per minute. One specimen was placed in fresh water and observed for five and one half hours. At the end of this time, the heart rate was 8 per minute.

Scott (3) observed Traube-Herring waves in blood pressure records made from pithed specimens of *Mustelus* and thought that they might be the result of the destruction of the spinal cord. They ceased when the animal was placed in fresh water. Greene (12) observed somewhat similar waves in the tracings made from the Chinook salmon but considered them to result from the rhythmical effects of the respiration on the blood pressure. Lyon (17) observed something similar to Traube-Herring waves in blood pressure tracings made from sharks in sea water.

Rhythmical variations in cardiac tone (tonus waves) were observed in *Squalus sucklii* but they were more prevalent while the fish were in tap water than with fish in sea water. They appeared to begin and sometimes to end with the gill cleaning movements. Respiratory waves were not observed in the heart records. (See figures, page 177.)

Changes in the Amount of Hemoglobin.—We determined the amount of hemoglobin in 44 fish before and after immersion in the experimental solutions. Meischer's modification of Fleischl's hæmoglobinometer was employed in making these determinations. This apparatus was calibrated for human blood and since it was not checked against any other method, the absolute amount of hemoglobin recorded may not be correct. However, the same method was employed for all the determinations and the relationship between them is therefore of value.

The results obtained from normal fish varied between 0.68 and 5.14 grams of hemoglobin per 100 cc. of blood with an average value of 2.84. We were unable to demonstrate any definite relationship between sex or weight of fish and the amount of hemoglobin. In an examination of the blood of the skate, Harris (26) found that the percentage of HbO_2 in the blood of this animal varied between 3.1 per cent. and 6.2 per cent. He considers that the average value for the normal skate is between 3.5 per cent. and 3.8 per cent. By the use of Oliver's tintometer, v. Fleischl's hæmometer or the spectrocolorimetric method of Rollet he obtained results which were in close agreement.

The average changes in amount of hemoglobin shown in Table II. are in close agreement with the differences in osmotic pressure which the fish encountered when transferred to the abnormal media. They appear to result largely from the dilution or concentration of the blood which followed the change in osmotic pressure of the external medium. However, it has been observed by Hall, Grey and Lepkovsky (27) that in the menhaden asphyxia leads to an increase in the hemoglobin value. A similar change may have been operating in *Squalus sucklii*.

Changes in Weight.—When the impermeability of the gills is decreased as the result of introduction of a fish into a solution having a salinity to which it is unaccustomed, water and salts would enter or leave the blood and tissue of the fish depending on the direction of the difference in the osmotic pressure. The direction and extent of the exchange of water might be indicated by a study of the weights of fish before and after the change in the external media had been made. It has frequently been observed that teleosts gain in weight when in a medium having a lower osmotic pressure than normal and lose weight in a hypertonic solution, Greene (12), Portier and Duval (28).

However, it was found by Gueylard and Portier (29) that sticklebacks "unlike any other fish" gain in weight in hypertonic and lose weight in hypotonic solutions, while Sumner (30) and Scott (31) observed that fish frequently gained but later lost in weight in the same solution and Scott especially noted that the changes in weight varied greatly for individual fish.

Regarding the average changes in weight of *Squalus sucklii* recorded in Table II.: fish in distilled water, tap water and tap water with a pH of 8.4 made a gain in weight which may be ascribed to the greater osmotic pressure of the blood than of the environment. The fish in tap water gained 3.66 per cent., a result which agrees well with the observation of Scott (3) that the average gain in water in a number of the tissues of *Mustelus* after immersion in fresh water was 3.1 per cent. The fish in distilled water gained less than those in tap water a result which may be explained by the fact that the latter fish lived on an average of 40 minutes longer in the tap water than did the fish in distilled water. The fish in fresh water pH 8.4 made a much greater gain in weight than did the others although they remained in the medium only a few minutes longer than the fish in distilled water. The large gain may be taken as indicative of an extensive injury to the gill membranes by the alkaline solution. The osmotic pressure of the solution containing NaCl, $CaCl_2$, KCl and $MgSO_4$ would be about that of sea water. Practically no change in weight resulted in *Squalus* placed in this solution. The osmotic pressure of the solution of NaCl, $CaCl_2$, KCl; of NaCl, $CaCl_2$; and of NaCl is each progressively lower than sea water. A loss of weight was observed in fish placed in each of these solutions. Fish placed in sea water to which salts had been added gained in weight, although the osmotic pressure of this solution was greater than that of the blood.

In general, the length of time fish were able to live in a solution was not related to the change in the weight of the fish. These results as well as those of other investigators previously mentioned indicate that a study of the changes in weight gives results which are difficult to interpret. The amount of water and salts passing through the gill membranes and the amount of these substances eliminated by the kidneys should largely determine the change in weight of a fish placed in an abnormal medium. Marked individual variations of these two factors may explain the peculiar results noted.

Variation in Weights of Liver, Spleen and Pancreas with the Weight of the Fish.—The weight per gram of fish of the liver, the spleen and the pancreas taken from fish that had died in sea water varies with the weight of the fish. Although individual fish frequently show considerable variations in the relative weights of these organs, when an average is taken of the results from a number of fish with approximately the same body weight, a rather definite relationship becomes evident (Table III.).

In fish taken dead from sea water the average weight of the liver per gram of fish rises rather rapidly from 0.066 for fish weighing between 300 and 999 grams to 0.115 for fish with weights between 3,000 and 3,999 grams. As larger fish are considered, the figure tends to remain comparatively constant; that for fish weighing between 6,000 and 6,999 grams being 0.117.

With the spleen, the greatest weight per gram of fish (0.0040) occurs in fish with a body weight between 300 and 999 grams. In larger fish, the figure falls in a rather regular manner until with fish having a total weight between 6,000 and 6,999 grams, the weight of the spleen per gram of fish is 0.0015.

The pancreases taken from fish that had died in sea water show a variation similar to that noted in the case of the spleens. With fish whose weight lay between 300 and 999 grams, the weight of the pancreas per gram of fish was 0.00286, and for fish weighing between 6,000 and 6,999 grams, the figure is 0.00142.

Pregnancy or sex did not appear to result in an alteration in the weight of the organs and therefore in this connection, these factors may be neglected. Insufficient data, however, is at hand to permit the formulation of an opinion as to whether the increased weight of the fish due to the presence of embryos was balanced by a compensatory decrease in the weight of the body of the fish or an increase in the weight of the organs. Since the weights recorded were obtained from fish captured only during

TABLE III.

AVERAGE WEIGHT OF ORGANS PER GRAM OF FISH.

Organ.	From Fish that Died in	Fish with Body Weight between (gm.)							
		300–999	1,000–1,499	1,500–1,999	2,000–2,999	3,000–3,999	4,000–4,999	5,000–5,999	6,000–6,999
Liver	Sea water	0.0660^{44}	0.0769^{9}	0.0940^{6}	0.1104^{22}	0.1156^{8}	0.0984^{5}	0.1235^{6}	0.1176^{4}
	Distilled H_2O	0.06324^{16}			0.1213^{2}		0.103^{2}	0.0826^{2}	
	Tap water	0.0602^{9}	0.0756^{7}	0.113^{3}	0.114^{1}	0.124^{3}	0.0897^{7}	0.0824^{8}	
	Tap water pH 8.4				0.1114^{2}	0.134^{1}			
	NaCl solution	0.0740^{4}	0.0664^{1}		0.0904^{1}	0.0468^{1}	0.110^{1}	0.0814^{1}	
	NaCl, $CaCl_2$ solution			0.1027^{2}	0.1246^{1}	0.0984^{1}			
	NaCl, $CaCl_2$, KCl solution	0.0704^{5}			0.1140^{1}				0.0894^{1}
	NaCl, $CaCl_2$, KCl, $MgSO_4$ solution	0.0658^{2}	0.0556^{1}	0.0880^{1}	0.1155^{2}				
	Sea water with added salts	0.0479^{3}	0.0927^{1}	0.0637^{1}			0.0971^{1}		
Spleen	Sea water	0.00407^{44}	0.00416^{9}	0.00345^{6}	0.00275^{22}	0.00271^{8}	0.00203^{5}	0.00167^{6}	0.00153^{4}
	Distilled H_2O	0.00475^{16}	0.00418^{2}		0.00300^{2}		0.00289^{2}	0.00189^{2}	
	Tap water	0.00439^{9}	0.00365^{7}	0.00326^{3}	0.00311^{1}	0.00214^{3}	0.00244^{7}	0.00231^{8}	
	Tap water pH 8.4	0.00312^{1}			0.00325^{2}	0.00261^{1}			
	NaCl solution	0.00355^{4}	0.00606^{1}		0.00357^{1}	0.00276^{1}	0.00238^{1}	0.00206^{1}	
	NaCl, $CaCl_2$ solution			0.00349^{2}	0.00254^{1}	0.00189^{1}			
	NaCl, $CaCl_2$, KCl solution	0.00390^{5}			0.00232^{1}				0.00163^{1}
	NaCl, $CaCl_2$, KCl, $MgSO_4$ solution	0.00439^{2}	0.00324^{1}	0.00346^{1}	0.00296^{2}				
	Sea water with added salts	0.00374^{3}	0.00321^{1}	0.00300^{1}			0.00140^{1}		

TABLE III. (*continued*).

Organ.	From Fish that Died in	Fish with Body Weight between (gm.)							
		300–999	1,000–1,499	1,500–1,999	2,000–2,999	3,000–3,999	4,000–4,999	5,000–5,999	6,000–6,999
Pancreas	Sea water	0.00286^{44}	0.00253^{9}	0.00224^{6}	0.00199^{22}	0.00167^{8}	0.00170^{5}	0.00139^{6}	0.00142^{4}
	Distilled H_2O	0.00337^{16}	0.00248^{2}		0.00211^{2}		0.00173^{2}	0.00145^{2}	
	Tap water	0.00324^{8}	0.00240^{7}	0.00203^{3}	0.00296^{1}	0.00182^{3}	0.00155^{7}	0.00142^{8}	
	Tap water pH 8.4	0.00239^{1}			0.00266^{2}	0.00258^{1}			
	NaCl solution	0.00319^{4}	0.00305^{1}		0.00236^{2}	0.00136^{1}	0.00170^{1}	0.00165^{1}	
	NaCl, $CaCl_2$ solution			0.00266^{2}	0.00198^{1}	0.00150^{1}			
	NaCl, $CaCl_2$, KCl solution	0.00299^{5}			0.00171^{1}				0.00093^{1}
	NaCl, $CaCl_2$, KCl, $MgSO_4$ solution	0.00266^{1}	0.00244^{1}	0.00242^{1}	0.00185^{2}				
	Sea water with added salts	0.00243^{3}	0.00227^{1}	0.00242^{1}			0.00140^{1}		

A fish in tap water, weight between 7,000–7,999, weight per gram of fish; liver 0.103, spleen 0.00153, pancreas 0.00099.
Small figures indicate the number of fish used.

the summer months, nothing can be said regarding the occurrence of seasonal variations in the weights of the organs.

With fish that had died in the experimental solutions, the weight of the fish after cessation of respiration was used in calculating the weight per gram of fish for the organs examined. Although possible sources of error in the method of determining the weights of the organs per gram of fish may be pointed out, the methods used in determining these values for fish that had died in the experimental solutions are so nearly the same, that a comparison of the figures is certainly of value. In the case of fish dying in sea water and also in the case of fish dying in the experimental solutions the results would probably have been more uniform if a much larger series of fish had been available. In general the weights of organs taken from the two classes of fish were in close agreement. Weights of organs taken from fish that had died in distilled water usually did run *slightly* higher than those taken from fish that had died in sea water, while these latter weights in turn were usually higher than those obtained from fish that had died in sea water with added salts.

Gueylard (32) working with a fresh water teleost, the stickleback, found the normal weight of the liver to be 0.0435 grams per gram of fish but after a 24-hour sojourn in water containing 20 grams per liter of NaCl, the weight had decreased to 0.0420.

A marine fish such as *Squalus* might be expected to show an increase in the weight of the liver after being placed in distilled water and in tap water but the results obtained show that this is not the case.

It is the contention of Gueylard that a relationship exists between the weight of the spleen and the resistance of fish to changes in salinity. This investigator has found the weight of the spleen per gram of fish in four species of marine teleosts to average from 0.0005 to 0.00073, while the spleen of five species of fresh water teleosts ranges from 0.00065 to 0.0024. Since the figures for the dogfish which had died in sea water range from 0.00407 to 0.00153, this fish appears to have a weight of spleen corresponding to that of the fresh water teleosts.

In a study of the stickleback, a fish readily adaptable to changes in salinity, Gueylard (32) found the weight of the spleen per gram of fish to be 0.00535 while the average figure for five less adaptable species of fresh water teleosts was 0.00065–0.0027. She has further stated that if the stickleback were placed in water of its natural habitat to which 20 grams of NaCl per liter has been added, the weight of the spleen per gram of fish at the end of 15 minutes was 0.00356 and at the end of two hours 0.00266. Somewhat similar changes were observed when eels were transferred from fresh water to sea water (33).

Introduction of a marine fish into fresh water might therefore, be expected to produce an increase in the weight of the spleen. The fact that this change was not observed with *Squalus sucklii* may be taken as an indication that a relationship exists between the inability of the dogfish to make a change in the weight of the spleen and its lack of adaptability to changes in salinity. A second hypothesis is that in *Squalus*, the change in weight of the spleen occurs slowly and has not progressed sufficiently before death takes place to make itself apparent. Neither of these explanations appears satisfactory. Gueylard (32) also reports that when sticklebacks were placed in water to which NaCl had been added, the spleen became flabby and the color which normally was brownish red had changed to reddish yellow. No changes in the color or consistency were observed by the author when the spleens taken from dogfish that had died in sea water were compared with those taken from the experimental solutions.

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Summary.

1. An investigation has been made of the effect on *Squalus sucklii* of transference to the following media: (*a*) distilled water, (*b*) tap water, (*c*) tap water pH 8.4, (*d*) NaCl solution, (*e*) NaCl, $CaCl_2$ solution, (*f*) NaCl, $CaCl_2$, KCl solution, (*g*) NaCl, $CaCl_2$, KCl, $MgSO_4$ solution, (*h*) sea water to which NaCl, $CaCl_2$, KCl, $MgSO_4$ had been added.

2. The most toxic solution was sea water with added salts, the solution of NaCl, $CaCl_2$ and KCl was the least toxic.

3. Tap water had a relatively low toxicity, but tap water which had been given the same pH as sea water by the addition of NaOH was more toxic.

4. Cessation of respiration invariably occurred more readily than did failure of the heart.

5. It appears likely that respiratory failure is the cause of death of fish in abnormal media. This is believed to result from a depression of the respiratory center.

6. No variation which could be ascribed to pregnancy, size or sex was observed in the duration of respiration or heart beat, the change in amount of hemoglobin, weight of fish or of organs while in the experimental solutions.

7. Changes in the amount of hemoglobin closely paralleled the changes in the osmotic pressure of the external media.

8. An increase in weight usually but not invariably resulted from introduction of fish into hypotonic media.

9. It could not be shown that a change in the comparative weight of the liver, spleen or pancreas followed the introduction of the fish into an abnormal medium.

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